

AMENDMENTS TO THE CLAIMS

Claims:

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1. (Original) A fluorescent protein derived from Green Fluorescent Protein (GFP) or any functional analogue thereof, wherein the amino acid in position 1 preceding the chromophore has been mutated to provide an increase in fluorescence intensity.

2. (Original) A fluorescent protein according to claim 1, wherein the chromophore is in position 65-67 of the predicted primary amino acid sequence of GFP.

3. (Original) A fluorescent protein according to claim 1 resulting in an increased fluorescence in cells expressing said fluorescent protein when said cells are incubated at a temperature of 30°C or above 30°C, preferably at a temperature of from 32°C to 39°C, more preferably at a temperature of from 35°C to 38°C, and most preferably at a temperature of about 37°C.

4. (Original) A fluorescent protein according to claim 1, said protein being derived from *Aequorea victoria* or *Renilla reniformis*.

5. (Original) A fluorescent protein according to claim 1, wherein the amino acid F in position 64 of GFP or Y66H-GFP has been

substituted by an amino acid selected from the group consisting of L, I, V, A and G.

6. (Original) A fluorescent protein according to claim 1, wherein the amino acid F in position 1 preceding the chromophore has been substituted by L and the amino acids of the chromophore include SYG, SHG or TYG.

7. (Original) A fluorescent protein according to claim 1 and having the amino acid sequence of Fig. 3, Fig. 4 or Fig. 5 herein.

8. (Original) A fusion compound consisting of a fluorescent protein (GFP) according to claim 1, wherein said GFP is linked to a polypeptide.

9. (Original) A fusion compound according to claim 8 wherein the polypeptide is a kinase, preferably the catalytic subunit of protein kinase A, or protein kinase C, or Erk1, or a cytoskeletal element.

10. (Original) A nucleotide sequence coding for the Fluorescent Protein of claim 1.

11. (Original) A nucleotide sequence according to claim 10 selected from the sequences shown in Fig. 3, Fig. 4 and Fig. 5.

12. (Currently Amended) A DNA construct ~~costruct~~ comprising a suitable control region or regions and a nucleotide sequence according to claim 10, the sequence being under the control of the control region.

13. (Original) A DNA construct according to claim 12 being under the control of the native GFP promoter, or a mammal constitutive or regulatory promoter, a viral promoter, a yeast promoter, a filamentous fungi promoter, or a bacterial promoter.

14. (Original) A host transformed with a DNA construct according to claim 12.

15. (Original) A host according to claim 14 selected from the following: organisms and cells belonging to bacteria, yeast, fungi, protozoans and higher eucaryots.

16. (Original) A process for preparing a polypeptide, comprising cultivating a host according to claim 14 and obtaining therefrom the polypeptide expressed by said nucleotide sequence.

17. (Original) A process according to claim 16 wherein the expression of the nucleotide sequence is effected by the native GFP promoter.


18. (Previously presented) Use of the Fluorescent Protein of claim 1 in an *in vitro* assay for measuring protein kinase activity, or dephosphorylation activity, wherein said fluorescent protein in purified form is added to a biological sample, preferably a cell extract, and any change in fluorescence is recorded.

19. (Previously presented) Use of the host of claim 14 in an *in vivo* assay for measuring metabolic activity, preferably kinase activity and dephosphorylating activity.

20. (Previously presented) Use of the fluorescent protein of claim 1 as a reporter for gene expression in living cells.

21. (Previously presented) Use of the fluorescent protein of claim 1 for the simultaneous monitoring of more than one gene in living, intact cells.

22. (Previously presented) Use of two or more of the fluorescent protein of claim 1 as organelle or cell tags for simultaneous visualisation of organelle or cell processes in living cells.

 23. (Currently Amended) A fluorescent protein derived from ~~wile-type~~ wild-type Green Fluorescent Protein (GFP) comprising a chromophore having three amino acid residues selected from the group consisting of SYG, SHG, TYG and THG, and in which the amino acid at least at position 1 upstream from the chromophore is different from the amino acid at the corresponding position in the wild-type GFP amino acid sequence counting from the chromophore, such that the substituted fluorescent protein exhibits an increase in the intensity of its fluorescence at a temperature of 30°C or above when expressed in a host cell.

24. (Previously presented) A fluorescent protein according to claim 23, which further comprises an amino acid substitution involving one or more of the three amino acids residues comprising the chromophore.

25. (Previously presented) A fluorescent protein according to claim 23, wherein an amino acid selected from the group consisting

of Leu, Ile, Val, Gly and Ala is substituted for the amino acid residue at position 1 upstream from the chromophore.

26. (Previously presented) A fluorescent protein according to claim 23, which is further substituted in that a His residue is submitted for the second amino acid residue of the chromophore.

27. (Previously presented) A nucleic acid molecule comprising a nucleotide sequence encoding a fluorescent protein derived from wild-type Green Fluorescent Protein) GFP having a chromophore having three amino acid residues selected from the group consisting of SYG, SHG, TYG and THG, and in which the amino acid substitution at least at position 1 upstream from the chromophore is different from the amino acid at the corresponding position of the wild-type GFP amino acid sequence counting from the chromophore, such that the substituted fluorescent protein exhibits an increase in the intensity of its fluorescence at a temperature of 30°C or above, when expressed in a host cell.

28. (Previously presented) A nucleic acid molecule according to claim 27, wherein an amino acid selected from the group consisting of Leu, Ile, Val, Gly and Ala is substituted for the amino acid residue at position 1 upstream from the chromophore.

29. (Previously presented) A nucleic acid molecule according to claim 28, wherein His is substituted for the second amino acid residue of the chromophore.

Ex. 1
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30. (Previously presented) An expression vector comprising suitable expression control sequences operatively linked to a nucleic acid molecule according to claim 28.

31. (Previously presented) A recombinant host cell comprising an expression vector that comprises a suitable control sequence operatively linked to a nucleic acid molecule according to claim 28.

32. (Previously presented) A fusion compound comprising a protein of interest fused to the fluorescent protein of claim 23.

33. (Previously presented) A nucleic acid molecule comprising a nucleotide sequence encoding a protein of interest fused to a nucleotide encoding the fluorescent protein of claim 23.

34. (Previously presented) A method of detecting the expression of a protein of interest in a cell which method comprises:

Ex Cont.
i) introducing into a cell a nucleic acid molecule comprising a nucleotide sequence encoding a protein of interest fused to a nucleic acid sequence encoding the fluorescent protein of claim 23, said nucleic acid molecule being operatively linked and under the control of a suitable expression control sequence;

ii) culturing the cell under conditions suitable for the expression of said protein of interest; and

iii) detecting the expression of said protein of interest by measuring the fluorescent of said cell by optical means.

35. (Previously presented) A method of simultaneously monitoring the expression of two or more different proteins of interest in a cell which method comprises:

i) providing two or more different nucleic acid molecules wherein each said nucleic acid molecule comprises a nucleotide sequence encoding a protein of interest fused to a nucleotide sequence encoding the fluorescent protein of claim 23 and being operatively linked to and under the control of a suitable expression control sequence and wherein each said fluorescent protein emits at a different wavelength;

ii) culturing the cell under conditions suitable for the expression of said proteins of interest; and

iii) comparing the expression of said proteins of interest in said cell by measuring the fluorescence of each of said fluorescent proteins by optical means.

36. (Previously presented) A method of detecting the expression of a protein of interest in a cell which method comprises:

iv) introducing into a cell a nucleic acid molecule comprising a nucleotide sequence encoding a protein of interest fused to a nucleic sequence encoding the fluorescent protein of claim 27, said nucleic acid molecule being operatively linked and under the control of a suitable expression control sequence;

v) culturing the cell under the condition suitable for the expression of said proteins of interest; and

vi) detecting the expression of said protein of interest by measuring the fluorescence of said cell by optical means.

37. (Previously presented) A method of simultaneously monitoring the expression of two or more different proteins of interest in a cell which method comprises:

iv) providing two or more different nucleic acid molecules wherein each said nucleic acid molecule comprises a nucleotide sequence encoding a protein of interest fused to a nucleotide sequence encoding the fluorescent protein of claim 27 and being operatively linked to and under the control of a suitable expression control sequence and wherein each said fluorescent protein emits at a different wavelength;

v) culturing the cell under conditions suitable for the expression of said proteins of interest; and

vi) comprising the expression of said proteins of interest in said cell by measuring the fluorescence of each of said fluorescent proteins by optical means.

38. (New) The fluorescent protein according to claim 1, having the amino acid sequence of SEQ. ID. NO.:18.

39. (New) The fluorescent protein according to claim 1, having the amino acid sequence of SEQ. ID. NO.:20.

40. (New) The fluorescent protein according to claim 1, having the amino acid sequence of SEQ. ID. NO.:22.